

tissues (Gusta and Wisniewski, 2013; Nenko *et al.*, 2019). Resistance to winter temperatures primarily depends on the degree of tissue maturation and water content in them before the onset of winter dormancy (Nenko *et al.*, 2020). A key question in studying adaptation is assessing the potential genetically determined capabilities of organisms as responses to adverse environmental factors (Nenko *et al.*, 2017; Yegorov *et al.*, 2017).

In the Žepče area, the average annual air temperature is 10.7°C, so only varieties resistant to low winter temperatures and with shorter growing seasons can be cultivated there. These are primarily white wine varieties with earlier maturation epochs that require less thermal sum for their ripening (Kojić and Delić, 2009). Achieving stable high yields is limited by the impact of unfavorable environmental factors such as winter frost, especially after dry and hot summers, and spring frost following thawing (Nenko *et al.*, 2017).

To achieve appropriate yield and grape quality, it is essential to know the biological characteristics of the variety and, based on that, perform the appropriate mature pruning. If the first buds are fertile in a variety, short pruning is essential, as leaving a larger number of buds on the shoot results in a higher yield but poorer grape quality. Subsequently, the shoots will not mature sufficiently, reducing their resistance to low winter temperatures, which are a regular occurrence in continental conditions. To avoid bud freezing and ensure adequate yield and grape quality, it is crucial to perform the appropriate mature pruning (Delić, 2015). Other factors are directly related to weather conditions such as temperature, light, water scarcity, or mineral nutrition. Little is known about the quantitative effects of these factors, and understanding the variation in yield from year to year and managing it remains a challenge for perennial crops (Hanke *et al.*, 2007), especially for grapevine (*Vitis vinifera* L.), as recent studies have shown (Clingleffer, 2010; Keller, 2021).

In addition to genetic potential, the position of the bud on the shoot is the main factor affecting bud fertility: it increases from the base to the middle section and decreases again towards the tip of the shoot (Huglin and Schneider, 1998). Quantitative relationships between temperature and bud fertility have been established, and an optimal temperature range for the formation of flower cluster primordia has been defined ($20^{\circ}\text{C} < T < 35^{\circ}\text{C}$) (Vasconcelos *et al.*, 2009). Particularly valuable data about the variety are those that discuss the fertility of buds along the shoot (from the base to the top). They are significant due to the load distribution, the number of individual fruitful elements, etc. (Žunić and Matijašević, 2004).

The aim of this study was to examine the degree of freezing of winter buds in two white wine grape varieties, Malvasia Istriana and Garganega, in the Žepče area, as well as the fertility of the buds along the shoots of the examined varieties.

MATERIALS AND METHODS

Material: In early March 2022, samples of the grape varieties Malvasia Istriana and Garganega were taken from a test vineyard. Mature shoots of the examined varieties were collected from a production vineyard located in Goliješnica (44.47°N, 18.07°E,

H= 230 m), in the municipality of Žepče, using a random selection method. The training form of the vines in the vineyard is the Veronese pergola, and the planting distance is 3.5 x 0.7 m. The vineyard is in full fruit production and regular agricultural practices are applied. The climate is continental, with a regular occurrence of low winter temperatures during the dormancy period. From each variety, 10 mature shoots were collected, with each shoot having 10 buds.

Method of Bud Provocation: This method is used to determine the degree of freezing of buds in winter shoots and to quickly obtain data on bud fertility for planning pruning. During the dormancy period, representative samples of 10 shoots with 10 buds each are taken. The shoots selected are those that would be left as fruiting elements during mature pruning. The shoots are cut into cuttings with one bud each, leaving an internode length of about 5 cm below the bud, and then placed in openings on a Styrofoam plate that has 10 x 10 holes (Picture 1). The plate with the cuttings is placed in a water bath to float, in a well-lit room where the temperature is around 25°C. After 10–15 days, the buds are activated, and young shoots appear. When these reach a length of about 15–20 cm, the flower clusters are observed, and their count is conducted according to the bud's position on the shoot (Picture 2,3). This way, the degree of freezing of the buds and the fertility of the buds along the shoots are determined. Based on these data, recommendations for pruning and yield planning are provided (Cindrić *et al.*, 2000; Žunić and Garić, 2010, cited by Briza and Milosavljević, 1955, 1958; Mijatović *et al.*, 2016).

Analysis of Results: A two-factor analysis of variance (ANOVA) was performed. The obtained data were processed using the SPSS software (IBM SPSS Statistics). The standard error was determined using the LSD test at a significance level of 0.05.

Ecological Conditions

For the analysis of meteorological conditions at the research site, Goliješnica in the municipality of Žepče, data from the meteorological station JUKIĆ – IEPE13 located in the test vineyard were used. The parameters were monitored for the year 2021, as the buds used in this study were formed during that growing season (Table 1).

Temperature extremes were also in line with the characteristics of a continental climate. The coldest month was January (1.8°C), while the warmest was July (22.6°C).

Table 1. Air temperature for 2021 years in Goliješnica, Žepče

Air temperature (°C) 2021 year			
Month	Maximum	Minimum	Average
I	15.2	-11.7	1.8
II	22.7	-12.1	4.6
III	20.7	-8.2	4.6
IV	26.3	-4.7	8.0
V	30.2	0	14.7
VI	38.0	4.2	20.0
VII	38.2	10.7	22.6
VIII	37.8	6.2	20.0

IX	32.7	2.2	15.6
X	27.7	-2.3	8.7
XI	24.6	-2.2	6.7
XII	16.2	-7.5	3.2
Mean annual average temperature			10.9
Mean annual vegetative temperature			15.7
Absolute maximum air temperature	38.2		
Absolute minimum air temperature		-12.1	

Based on the presented data (Table 2), we see that the relative humidity in the Žepče area during 2021 was lowest in May (78%), while the highest was recorded in November (95%), leading us to conclude that relative humidity was high throughout the year studied. The average annual relative humidity during 2021 in the test vineyard was measured at 84%.

Table 2. Relative air humidity during 2021 year

Relative air humidity													
Month	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Annual average
%	90	82	79	79	78	79	80	82	85	93	95	91	84

RESULTS AND DISCUSSION

The results of the analysis of the freezing degree and bud fertility along the shoots of the Malvasia Istriana and Garganega varieties at the Goliješnica site in the municipality of Žepče are presented in the tables.

Freezing degree of winter buds



Picture 1. Cuttings of the Malvasia Istriana and Garganega varieties in the water bath (orig.)

From the data in Table 3, we can see that the Malvasia Istriana variety showed no freezing of the buds, and all buds were activated. Therefore, the planned mature pruning can be applied without any adjustments.

Table 3. Register activated winter buds in the Malvasia Istriana variety

Shoots	Ordinal number winter buds along the shoots									
	1	2	3	4	5	6	7	8	9	10
I	+	+	+	+	+	+	+	+	+	+
II	+	+	+	+	+	+	+	+	+	+
III	+	+	+	+	+	+	+	+	+	+
IV	+	+	+	+	+	+	+	+	+	+
V	+	+	+	+	+	+	+	+	+	+
VI	+	+	+	+	+	+	+	+	+	+
VII	+	+	+	+	+	+	+	+	+	+
VIII	+	+	+	+	+	+	+	+	+	+
IX	+	+	+	+	+	+	+	+	+	+
X	+	+	+	+	+	+	+	+	+	+

In the case of the Garganega variety (Table 4), there was freezing in 10 out of a total of 100 buds, but this is a small percentage, so no adjustments to the mature pruning need to be made. Analyzing the obtained results, we can conclude that the ecological conditions in the Goliješnica area, municipality of Žepče, are more favorable for the Malvasia Istriana variety compared to the Garganega variety when considering the indicator of the degree of freezing of winter buds.

Table 4. Register activated winter buds in the Garganega variety

Shoots	Ordinal number winter buds along the shoots									
	1	2	3	4	5	6	7	8	9	10
I	-	+	+	+	+	+	+	+	+	+
II	+	+	+	+	+	+	+	+	+	+
III	+	+	+	+	+	+	+	-	-	+
IV	+	+	+	+	+	+	+	+	+	+
V	+	-	+	+	+	+	+	+	+	+
VI	+	+	+	+	+	+	+	+	+	+
VII	+	+	+	+	+	+	+	+	+	+
VIII	-	+	+	+	-	+	+	+	+	+
IX	+	+	+	+	+	+	+	+	+	+
X	-	+	+	-	+	+	+	+	-	-

The resistance of winter buds to low temperatures depends, to a large degree, on their position on the shoot. Many studies have shown that basal buds have a higher tolerance to low winter temperatures than middle and apical buds (Köse and Kaya, 2017). In grape, the tolerance of winter buds to low temperatures is directly related with maturation cane (Fennell, 2004).



Pictures 2 and 3. Grown shoots with flowering in the Malvasia Istriana and Garganega varieties (orig.)

Fertility of the Examined Grape Varieties

Fertility, as a biological property of grape varieties, is expressed through the following indicators:

- Á Percentage of activated (germinated) buds,
- Á Number of developed shoots,
- Á Number of fruiting shoots,
- Á Number of flower clusters,
- Á Fertility coefficients (potential, relative, and absolute).

The fertility indicators are influenced by the biological characteristics of the variety, the position of the bud on the shoot, ecological conditions, and applied ampelotechnics. The division into varieties of low, medium, and high fertility is primarily the result of their fruiting potential, but also of their ability to adapt to different cultivation systems, technological interventions (load, fertilization, irrigation), and adaptability to climatic and pedological conditions in various areas (Maletić *et al.*, 2008).

Percentage of Activated Buds is calculated using the formula: $Pko = B \times 100 / A$, where B is the number of total developed shoots, and A is the number of retained buds.

$Pko = 114 \times 100 / 100$ (MI); $Pko = 92 \times 100 / 100$ (G)

From the obtained data, we can conclude that the percentage of activated buds for the variety Malvasia Istriana in the Žepče area was 114%. The percentage of activated buds for the variety Garganega was 92%, as not all retained buds developed into shoots. This is a high percentage of activated buds, so there is no need to adjust the pruning.

Total number of developed and fruiting shoots and number of flower clusters During the growing season, green shoots, known as shoots, form from the buds on the vine. The number of developed shoots on the vine from the retained buds during pruning has a direct impact on the fertility of the variety (Delić, 2010).

For the variety Malvasia Istriana (114), a statistically significantly larger number of total shoots developed compared to the variety Garganega (92). From a certain number of buds, shoots developed, including from latent buds, resulting in a greater number of developed shoots than the number of activated buds. The difference in the number of fruiting shoots between the varieties Malvasia Istriana (103) and Garganega (90) was not statistically significant. The number of flower clusters for the variety Malvasia Istriana (126) was statistically significantly higher compared to the variety Garganega (95) under the same ecological conditions (Table 5).

Table 5. Total number of developed and fruiting shoots and number of flower clusters developed from 10 winter buds of Malvasia Istriana and Garganega varieties

Shoots	Total number developed shoots		Number fruiting shoots		Number of flowers	
	MI	G	MI	G	MI	G
I	10	9	10	9	11	10
II	10	10	9	10	13	12
III	10	8	10	8	12	8
IV	12	11	9	10	10	10
V	11	9	11	9	14	9
VI	12	10	11	10	15	9
VII	10	10	8	10	11	12
VIII	14	8	13	8	13	9
IX	13	11	13	10	16	10
X	12	6	9	6	11	6
Total	114 ^a	92 ^b	103	90	126 ^a	95 ^b
	LSD test $_{0.05}=1.252$		ns		LSD test $_{0.05}=1.754$	

MI – Malvasia Istriana, G – Garganega; ns – no significant

The formation of yield in grapevines begins with the development of flower clusters in latent buds during the season 1. Unlike other perennial crops, there is no evidence of competition in grapevines between, on one hand, the initiation and differentiation of flower clusters for the following season and, on the other hand, the development of flowers and fruit set for the current season (Vasconcelos *et al.*, 2009).

According to the data in Table 6, we can see that a greater number of flower clusters developed in buds from the 5th to the 10th node on the shoots of the Malvasia Istriana variety, while for the Garganega variety, the highest number of flower clusters was formed in buds developed at the 6th and 7th nodes on the shoot. Statistically significant differences in the number of formed flower clusters based on the position of the bud on the shoot for the Malvasia Istriana and Garganega varieties were recorded only for buds

developed at the 7th, 9th, and 10th nodes. Differences in the number of flower clusters developed in buds at all other nodes for the examined varieties were not statistically significant.

Table 6. Fertility of winter buds depending on the position on the shoot

Ordinal number of winter buds on the shoot	Number of flowers		Significance of the differences
	MI	G	
1	9	9	Ns
2	8	9	Ns
3	11	10	Ns
4	10	9	Ns
5	12	9	Ns
6	14	12	Ns
7	16	11	LSD test $_{0.05} = 0.401$
8	15	9	Ns
9	17	9	LSD test $_{0.05} = 0.49$
10	14	8	LSD test $_{0.05} = 0.44$
Ukupno	129	95	

MI – Malvasia Istriana, G – Garganega; ns – no significant

Malvasia Istriana has high economic value as it combines good fertility with high-quality grapes and wine, and in addition, it has a pronounced typicality and recognizability related to the areas of Istria and its surroundings. Its fertility is generally regular; however, it can sometimes be irregular due to pollination issues, which can occur when rain and cold weather happen during flowering (Maletić et al., 2015). The fertility of the Garganega variety is regular and average. It suits high cultivation systems and mixed pruning. It is sensitive to low winter temperatures (Mirošević and Turković, 2003).

Fertility coefficients

Table 7. Fertility coefficients of the tested varieties

Variety	Fertility coefficients of winter buds	Relative fertility coefficients	Absolute fertility coefficients
Malvasia Istriana	1.26	1.1	1.2
Garganega	0.95	1.03	1.05

The Malvasia Istriana variety had higher values for all three fertility coefficients compared to the Garganega variety (Table 7) in this study. According to the value of the fertility coefficient, both varieties fall into the group of varieties with a high relative fertility coefficient (0.9 – 1.1) and into the group with a very low absolute fertility

coefficient (<1.2) (Žunić and Garić, 2010; Matijašević, 2021). In studies conducted in Radmilovac, the Malvasia Istriana variety had the following coefficients: bud fertility 1.23, fertility 1.36, and productivity 1.62, while those grown in Niš had these coefficients: bud fertility 0.93, fertility 1.06, and productivity 1.2 (Sivčev *et al.*, 2003).

CONCLUSIONS

Investigation of the degree of winter bud freezing and fertility of the Malvasia Istriana and Garganega varieties in the Žepče area during 2022 showed that both varieties can be successfully cultivated under the given agroecological conditions. Greater resistance and fertility were evident in the Malvasia Istriana variety compared to the Garganega variety. Further research should be conducted to confirm the obtained results.

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STEPEN IZMRZAVANJA I RODNOST ZIMSKIH OKACA SORTI VINOVE LOZE MALVAZIJA ISTARSKA I GARGANEGA NA PODRUČJU ŽEPČA

Rezime

Cilj ovog istraživanja je bio ispitati stepen izmrzavanja i rodnost zimskih okaca duž lastara vinskih sorti vinove loze Malvazija istarska i Garganega gajenih na području Žepča. Vinova loza je osjetljiva na niske zimske temperature, a stepen izmrzavanja zimskih okaca i drugih organa zavisi od bioloških osobina sorte i ekoloških uslova gajenja. Stepen izmrzavanja zimskih okaca utvrđen je metodom provokacije okaca, dok je rodnost okaca utvrđena brojanjem cvasti po okcu, lastaru i rodnom lastaru. Uzorci su uzeti početkom marta, po 10 lastara sa po 10 okaca za svaku sortu. Praćeni su rodnost i stepen izmrzavanja okaca po dužini lastara. Na osnovu dobijenih rezultata izračunati su procenti krenulih okaca, koeficijenti rodnosti okaca i lastara, te plodnosti sorti, kao i rodnost razvijenih okaca od prvog do desetog koljenca na lastaru. Analizom izmjerenih parametara možemo zaključiti da se ispitivane sorte mogu uspješno gajiti u ekološkim uslovima Žepča, a vrijednosti svih ispitivanih parametara su bile veće kod sorte Malvazija istarska u odnosu na sortu Garganega.

Ključne riječi: *Malvazija istarska, Garganega, okca, rodnost, izmrzavanje*